

Elevated PCB Levels in Puget Sound Harbor Seals (*Phoca vitulina*)

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Introduction

Environmental contaminants including the polychlorinated-biphenyls (PCBs), -dibenzo-*p*-dioxins (PCDDs or dioxins) and -dibenzofurans (PCDFs or furans), persist in environmental compartments as a result of their chemical stability, and bioaccumulate in the food chain because of their fat-soluble nature. While the input of PCDDs and PCDFs into the aquatic environment has primarily been the result of incomplete combustion or as by-products of various industrial processes, the input of PCBs largely reflects the leakage and improper disposal of these manufactured heat-resistant oils. Despite restrictions on their use in the 1970s, PCBs continue to be found at high levels in wildlife species at the top of aquatic and marine food chains (Tillitt et al., 1992; Muir et al., 1992).

Harbor seals (*Phoca vitulina*) have been found to have high levels of contaminants in many parts of the world, including the German Wadden Sea (Skaare et al., 1990), the Baltic Sea (Blomkvist et al., 1992), the United Kingdom (Hall et al., 1992) and central California (Kopec and Harvey, 1995). However, accurate comparisons of results from different studies are difficult, owing to variable sample quality (dead vs. live animals; trauma or illness as cause of death; state of decomposition; age; sex) and the numerous analytical techniques used. When interpreted cautiously, a comparison of different results can be useful as a means of identifying relative degrees of contamination in different harbor seal populations.

Studies carried out in the Netherlands found that captive harbor seals fed herring from the highly contaminated Baltic Sea exhibited immunotoxicity and retinoid disruption when compared to seals fed herring from the relatively uncontaminated Atlantic Ocean (De Swart et al., 1996; Ross et al., 1996a; Ross et al., 1996b). As a result, it is now thought that elevated levels of organochlorine chemicals may have contributed to the severity of the 1988 virus-associated mass mortality of 20,000 harbor seals in northern Europe (De Swart et al., 1995a; Osterhaus et al., 1995; Ross et al., 1996a). In addition, reduced plasma levels of vitamin A and thyroid hormone (De Swart et al., 1995b; Reijnders, 1986), and impaired reproduction (Reijnders, 1986) were observed in captive harbor seals fed contaminated fish.

Because of their high trophic position in the marine ecosystem and their wide geographical distribution, harbor seals can serve as a sentinel species for environmental contamination. It is estimated that approximately 17,000 harbor seals inhabit the inland waters of Washington State, stretching from Port Angeles in the Juan de Fuca Strait, to the southern tip of Puget Sound (Jeffries et al., 1997). Of these, about 1,800 individuals are found in Puget Sound proper (Jeffries et al., 1997). Adult harbor seals in these areas undergo little in the way of migration, and are considered year-round residents of these waters. While harbor seals prefer the lipid-rich herring (*Clupea harengus*) and hake (*Merluccius productus*) as prey items (Olesiuk, 1993), they are opportunistic, and dependent upon local availability, will feed on a wide variety of fish and invertebrate species. Harbor seals in Puget Sound have been found to be highly omnivorous, with 75% of their estimated average annual diet made up of Pacific cod (*Gadus macrocephalus*), Pacific herring, English sole (*Parophrys vetulus*), Plainfish midshipman (*Porichthys notatus*), and Pacific hake (S. Jeffries, unpublished observations). The complexity of the Puget Sound harbor seal diet may reflect the low abundance of hake and herring compared to the Strait of Georgia, with seals consuming a wide selection of prey items (see Figure 1).

The Puget Sound Food Chain

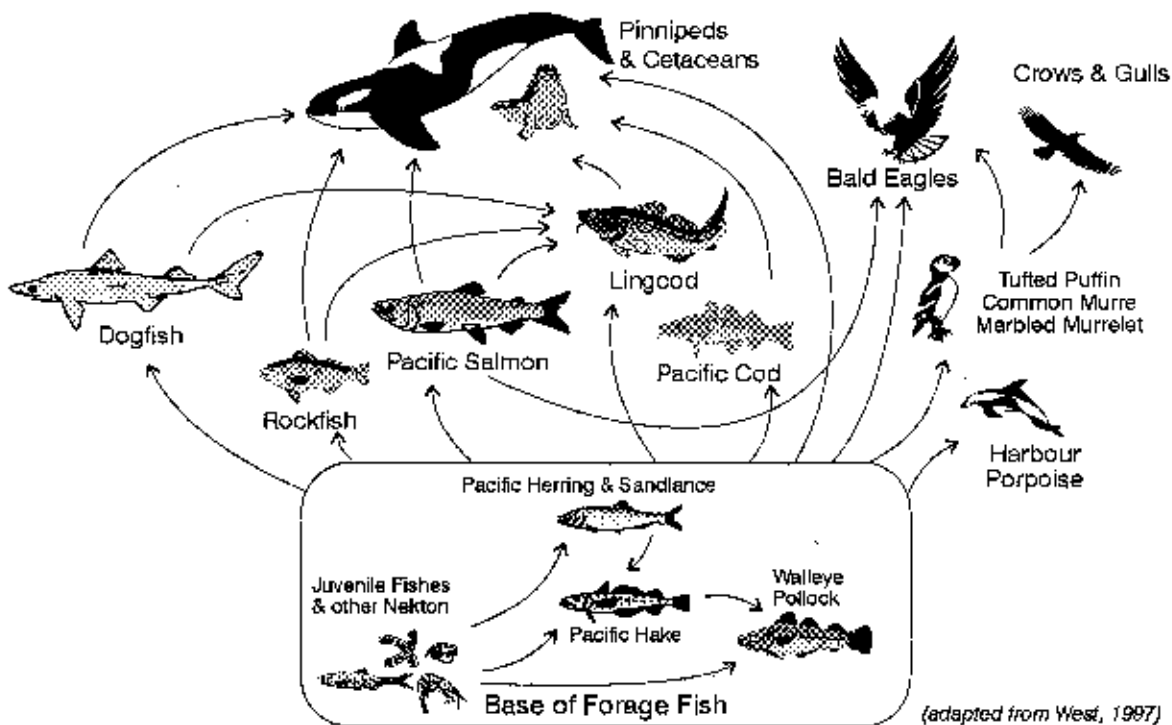


Figure 1. Harbor seals occupy a high trophic level in the Puget Sound ecosystem, and consume a wide variety of different prey species (adapted from West, 1997).

Previous studies have found that harbor seals (Hong et al., 1996; Calambokidis et al., 1991) and other marine mammals (Jarman et al., 1996; Calambokidis et al., 1985) frequenting Puget Sound and adjacent coastal waters of Washington State have high levels of contaminants. While these studies suggest that a regional contamination problem exists, differences in analytical techniques make it difficult to compare these data directly to the results obtained in other areas, or over time.

In order to describe the current levels of PCBs and related PCDDs and PCDFs in Puget Sound harbor seals, we collected blubber samples by biopsy from harbor seal pups on Gertrude Island in 1996. Samples were analyzed by high-resolution gas chromatography-mass spectrometry for all 2,3,7,8-chlorine substituted PCDDs and PCDFs (n=17), as well as all PCB congeners (n=209).

Methods

Blubber samples were obtained from healthy, young harbor seals (n=17), estimated to be four weeks of age, during the breeding season of 1996, from Gertrude Island in south Puget Sound (see Figure 2). Samples were collected by biopsy from live pups caught by beach seine at Gertrude Island. Briefly, a 6-mm disposable biopsy punch was used to obtain a core of skin and blubber (approximately 250 mg) under aseptic conditions from a lateral site above the left hip of the animal. All samples were placed in aluminum foil and stored at -20 °C until analysis.

Approximately 100–200 mg of blubber was used for congener-specific determination of PCBs, PCDDs, and PCDFs at the Regional Dioxin Laboratory (Institute of Ocean Sciences). Blubber samples were homogenized unfrozen and spiked with a mixture of ¹³C-labeled PCDD/Fs non-ortho, mono-ortho and di-ortho substituted PCB (NO-, MO- and DO-PCB, respectively). The composition of the internal standard spiking solutions, sample extraction procedures, column chromatographic clean-up and carbon-

fiber fractionation of the extracts are described elsewhere (Rantaleinen et al., 1998; MacDonald et al., 1998; Ikonou, 1998). All fractions collected from the carbon-fiber system were concentrated to less than 10 μ L, spiked with the corresponding ^{13}C -labeled method performance standards and analyzed by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). For all analyses the MS was operated at 10,000 resolution under positive electron impact (EI) conditions and data were acquired in the Single Ion Resolving Mode (SIR). Details on the GC and MS conditions are described elsewhere for the PCDD/Fs, and the MO- and NO-PCBs (Rantaleinen et al., 1998; MacDonald et al., 1998) and the DO-PCBs (Ikonou, 1998). The criteria for identification and quantification and the quality control measures undertaken for the HRGC/HRMS analysis of all the analytes of interest were based on procedures established in the Environment Canada "River Road" protocol (Environment Canada, 1992b; Environment Canada, 1992a) for PCDD/PCDF analysis. The same criteria and quality assurance quality control procedures were also applied to the NO- MO- and DO-PCB analyses. PCB concentrations were calculated by adding the concentrations determined for all 209 congeners.

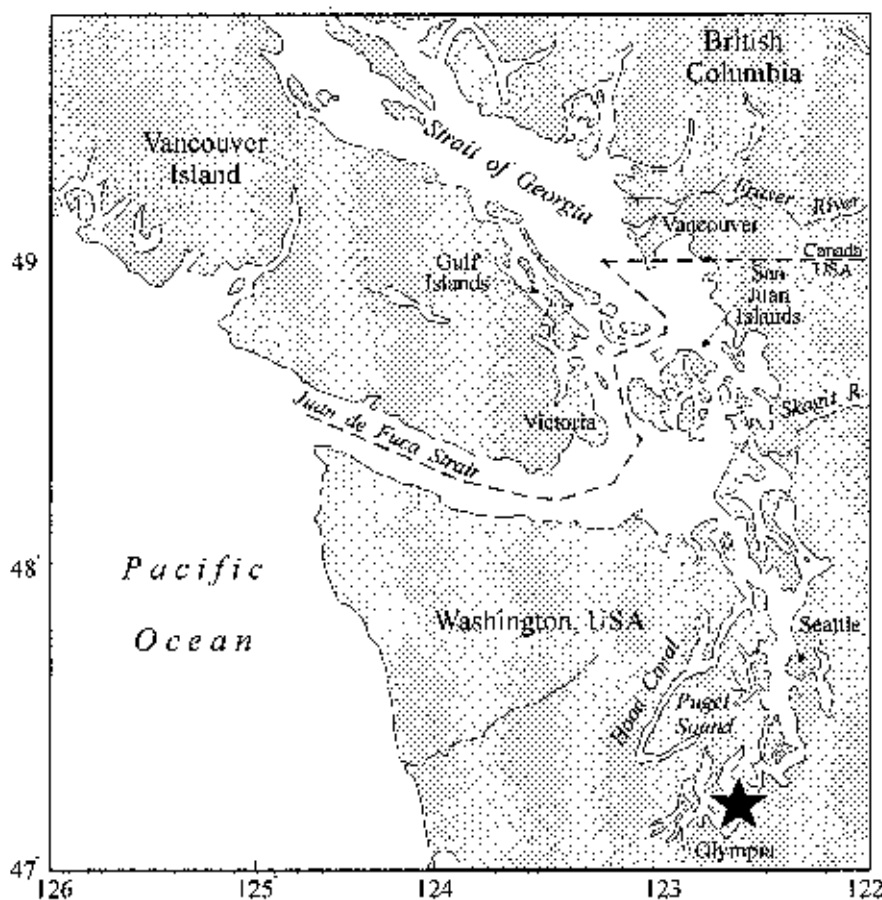


Figure 2. Blubber samples were collected by biopsy from young harbor seals on Gertrude Island in south Puget Sound.

Based on an extrapolation of the PCB-age relationship established for Strait of Georgia harbor seal males and females (Ross et al., 1997) to the mean PCB levels in Puget Sound harbor seal pups in 1996, we modeled PCB dynamics for the Washington State harbor seal population. For this, we used: 1) a correction factor developed by a comparison of mean PCB levels in pups from the Strait of Georgia and pups from Gertrude Island; 2) an extrapolation of the PCB-vs.-age relationship (equations of lines of best fit) established for the Strait of Georgia harbor seal males and females on the basis of this correction factor; 3) an assumption of a 30%

lipid content in harbor seals; and 4) an age distribution for males and females from a population model developed for Strait of Georgia harbor seals (Olesiuk, 1993). On this basis, we estimated the total amount of PCBs present in the approximately 1,763 harbor seals inhabiting Puget Sound proper.

Results and Discussion

Of the three classes of chemicals measured, PCBs represented the predominant contaminant in harbor seal blubber from Puget Sound in 1996, averaging 16.9 ± 2.90 mg/kg (lipid weight; mean \pm s.e.m.), compared to only very small amounts of PCDDs (141.5 ± 18.8 ng/kg lipid weight) and PCDFs (12.3 ± 1.5 ng/kg lipid weight). When the concentrations of dioxin-like PCBs, PCDDs and PCDFs are converted to total Toxic Equivalents (TEQ) to 2,3,7,8-TCDD, using international Toxic Equivalency Factors (TEFs) (Ahlborg et al., 1994; Van Zorge et al., 1989), PCBs remain an important contaminant, contributing approximately 95% to the total TEQ in blubber. PCDDs, including the most toxic congener, 2,3,7,8-TCDD, contributed approximately 5%, and PCDFs contributed 1% to the total TEQ. The total TEQ for dioxin-like PCBs, PCDDs, and PCDFs was 167.2 ± 27.3 ng/kg (lipid weight). This suggests that PCBs are not only present at very high concentrations relative to the dioxins and furans, but that they also present the greatest "dioxin-like" toxic risk to Puget Sound harbor seals.

Harbor seals represent an important predator at the top of the Puget Sound food chain. They bioaccumulate fat-soluble contaminants including PCBs, PCDDs, and PCDFs, as well as numerous pesticides, from the food chain, and can provide valuable information on the state of contamination of the Puget Sound ecosystem. An understanding of contaminant levels, patterns and trends in harbor seals is relevant to issues of wildlife management, ecosystem health, and human health.

While not conclusive, studies of free-ranging marine mammals have provided circumstantial evidence that ambient levels of environmental contaminants have adversely affected their well-being (see Table 1). The more recent captive-feeding studies involving harbor seals have provided more evidence that contaminants found in fish are immunotoxic and endocrine disrupting (De Swart et al., 1996; Ross et al., 1996a; Ross et al., 1996b). These results provide a means of identifying free-ranging populations of harbor seals or other marine mammals at possible risk.

Table 1: Epidemiological studies that have associated contaminant levels with adverse biological effects in different parts of the world.

Observation	Species	Location	Reference
abortions, premature pupping	California sea lion	California	DeLong et al., 1973
tumors, decreased fecundity	Beluga whale	St. Lawrence estuary	Martineau et al., 1987
impaired reproduction	ringed seals	northern Europe	Helle et al., 1976
skeletal lesions	harbor, grey seals	northern Europe	Mortensen et al., 1992; Bergman et al., 1992
reduced testosterone	Dall's porpoises	north Pacific Ocean	Subramanian et al., 1987
diminished T-cell responses	bottlenose dolphins	Gulf of Mexico	Lahvis et al., 1995

Age and sex represent factors that are important when assessing contaminant levels, since males accumulate fat-soluble contaminants throughout their lifetime, whereas females transfer a significant amount of their contaminant burden to their offspring (Aguilar and Borrell, 1994b; Addison and Brodie, 1987). Studies carried out in the Strait of Georgia indicate that such a relationship also exists for west coast harbor seals, with older males being the most contaminated, while older females are relatively uncontaminated (Ross et al., 1997).

On the basis of the PCB levels observed in Puget Sound pups, an extrapolation of the PCB relationship with age and sex observed in Strait of Georgia harbor seals (Ross et al., 1997), and of the age distribution model from the Strait of Georgia (Olesiuk, 1993), we estimated the total amount of PCBs in Puget Sound harbor seals to be 905 g. The estimated total PCB load of Puget Sound harbor seal males is

699 g, and for females is 206 g, reflecting the loss of these fat-soluble contaminants by females during reproduction and lactation.

Mean levels of PCBs in Puget Sound harbor seal pups were approximately three times higher than those observed in same-age animals inhabiting the Strait of Georgia, British Columbia (Ross et al., 1997). This suggests that the Puget Sound basin remains relatively contaminated with PCBs, in particular, and that the food chain continues to bioaccumulate environmental contaminants.

PCB concentrations observed in 1996 blubber samples from Puget Sound harbor seal pups are similar to the levels found to be immunotoxic in a captive feeding study in the Netherlands (De Swart et al., 1996; Ross et al., 1996a; Ross et al., 1996b), suggesting that contaminant levels continue to represent a tangible concern for this wildlife species. Immunotoxic contaminants are suspected to have played a role in exacerbating recent virus-associated mass mortalities among harbor seals in northern Europe (Osterhaus et al., 1995; De Swart et al., 1995a), striped dolphins in the Mediterranean Sea (Aguilar and Borrell, 1994a), bottlenose dolphins on the east coast of the United States (Kuehl et al., 1991), and Baikal seals in Lake Baikal (Nakata et al., 1995). As such, a potential risk exists for a contaminant-associated immunotoxicity among Puget Sound seals, something that may predispose these animals to a serious outbreak of infectious disease.

Concentrations of PCBs in many wildlife samples declined following the implementation of regulatory controls in the 1970s (Addison et al., 1986), although levels appear to have stabilized in many parts of the world since the mid-1980s (Loganathan et al., 1990). This suggests that PCBs continue to cycle in the environment, and that atmospheric inputs, leaking from former storage sites, and recycling from sediments and the food chain may be preventing a more rapid decrease in these contaminant levels.

This study suggests that PCB levels in young Puget Sound harbor seals are high enough to cause adverse biological effects. Future research should include:

1. continued monitoring of contaminant levels in Gertrude Island harbor seal pups (temporal trends);
2. assessment of contaminant levels in harbor seals inhabiting other inland and coastal waters of Washington State (spatial trends);
3. characterization of the relationship between contaminant levels and age in both male and female harbor seals;
4. research into the possible adverse effects of contaminants on Puget Sound harbor seals; and
5. research into congener-specific contaminant patterns (PCBs, PCDDs, and PCDFs) in the Puget Sound food chain in order to better track contaminant sources and flow through the food chain and identify major routes of contaminants to marine mammals and humans.

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References

- Addison, R.F., and Brodie, P.F. (1987) Transfer of organochlorine residues from blubber through the circulatory system in milk in the lactating grey seal, *Halichoerus grypus*. *Can.J.Fish.Aquat.Sci.* 44, 782.
- Addison, R.F., Zinck, M.E., and Smith, T.G. (1986) PCBs have declined more than DDT-group residues in Arctic ringed seals (*Phoca hispida*) between 1972 and 1981. *Environ.Sci.Technol.* 20, 253.
- Aguilar, A., and Borrell, A. (1994a) Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990-1992 Mediterranean epizootic. *Sci.Total.Envir.* 154, 237.

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- Aguilar, A., and Borrell, A. (1994b) Reproductive transfer and variation of body load of organochlorine pollutants with age in fin whales (*Balaenoptera physalus*). Arch.Environ.Contam.Toxicol. 27, 546.
- Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., Brouwer, A., Derks, H.J.G.M., Feeley, M., Golor, G., Hanberg, A., Larsen, J.C., Liem, A.K.D., Safe, S.H., Schlatter, C., Waern, F., Younes, M., and Yrjanheikki, E. (1994) Toxic equivalency factors for dioxin-like PCBs. Chemosphere 28, 1049.
- Bergman, A., Olsson, M., and Reiland, S. (1992) Skull-bone lesions in the baltic grey seal (*Halichoerus grypus*). Ambio 21, 517.
- Blomkvist, G., Roos, A., Jensen, S., Bignert, A., and Olsson, M. (1992) Concentrations of sDDT and PCB in seals from Swedish and Scottish waters. Ambio 21, 539.
- Calambokidis, J., Speich, S.M., Peard, J., Steiger, G.H., Cabbage, J.C., Fry, D.M., and Lowenstine, L.J. (1985) Biology of Puget Sound marine mammals and marine birds: population health and evidence of pollution effects. N.O.A.A. Technical Memorandum NOS OMA 18, Rockville, MD, USA.
- Calambokidis, J., Steiger, G.H., Lowenstine, L.J., and Becker, D.S. (1991) Chemical contamination of harbor seal pups in Puget Sound. U.S.E.P.A. 910/9-91-032.
- De Swart, R.L., Harder, T.C., Ross, P.S., Vos, H.W., and Osterhaus, A.D.M.E. (1995a) Morbilliviruses and morbillivirus diseases of marine mammals. Infect.Agent.Dis. 4, 125.
- De Swart, R.L., Ross, P.S., Timmerman, H.H., Van Loveren, H., Reijnders, P.J.H., Vos, J.G., and Osterhaus, A.D.M.E. (1995b) Impairment of immune function in harbour seals (*Phoca vitulina*) feeding on fish contaminated through the food chain. Hum.Exp.Toxicol. 14, 692.
- De Swart, R.L., Ross, P.S., Vos, J.G., and Osterhaus, A.D.M.E. (1996) Impaired immunity in harbour seals (*Phoca vitulina*) exposed to bioaccumulated environmental contaminants: review of a long-term study. Environ.Health Perspect. 104 (suppl. 4), 823.
- Delong, R.L., Gilmartin, W.G., and Simpson, J.G. (1973) Premature births in California sea lions: Association with high organochlorine pollutant residue levels. Science 181, 1168.
- Environment Canada (1992a) Internal quality assurance requirements for the analysis of dioxins in environmental samples. Supply and Services Canada.
- Environment Canada (1992b) Reference method for the determination of PCDDs and PCDFs in pulp and paper mill effluents. Supply and Services Canada.
- Hall, A.J., Law, R.J., Harwood, J., Ross, H.M., Kennedy, S., Allchin, C.R., Campbell, L.A., and Pomeroy, P.P. (1992) Organochlorine levels in common seals (*Phoca vitulina*) which were victims and survivors of the 1988 phocine distemper epizootic. Sci.Total.Envir. 115, 145.
- Helle, E., Olsson, M., and Jensen, S. (1976) DDT and PCB levels and reproduction in ringed seal from the Bothnian Bay. Ambio 5, 188.
- Hong, C.S., Calambokidis, J., Bush, B., Steiger, G.H., and Shaw, S. (1996) Polychlorinated biphenyls and organochlorine pesticides in harbor seal pups from the inland waters of Washington State. Environ.Sci.Technol. 30, 837.
- Ikonomou, M.G. (1998) Single congener PCB analysis by HRGC/HRMS: QA/QC measures for accurate determinations. Manuscript in preparation.
- Jarman, W.M., Nostrom, R.J., Muir, D.C.G., Rosenberg, B., Simon, M., and Baird, R.W. (1996) Levels of organochlorine compounds, including PCDDs and PCDFs, in the blubber of cetaceans from the West coast of North America. Mar.Pollut.Bull. 32, 426.
- Jeffries, S.J., Brown, R.F., Huber, H.R., and Delong, R.L. (1997) Assessment of harbor seals in Washington and Oregon, 1996. NOAA AFSC Processed Report 97-10, p. 83.
- Kopec, A.D., and Harvey, J.T. (1995) Toxic pollutants, health indices, and population dynamics of harbor seals in San Francisco Bay, 1989-1992. Moss Landing Marine Laboratories, Moss Landing, USA, p. 138 pp.
- Kuehl, D.W., Haebler, R., and Potter, C. (1991) Chemical residues in dolphins from the U.S. Atlantic coast including atlantic bottlenose obtained during the 1987/1988 mass mortality. Chemosphere 22, 1071.

- Lahvis, G.P., Wells, R.S., Kuehl, D.W., Stewart, J.L., Rhinehart, H.L., and Via, C.S. (1995) Decreased lymphocyte responses in free-ranging bottlenose dolphins (*Tursiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. *Environ. Health Perspect. Suppl.* 103, 67.
- Loganathan, B.G., Tanabe, S., Tanaka, H., Watanabe, S., Miyazaki, N., Amano, M., and Tatsukawa, R. (1990) Comparison of organochlorine residue levels in the striped dolphin from western north Pacific, 1978-79 and 1986. *Mar. Pollut. Bull.* 21, 435.
- MacDonald, D.D., Ikonou, M.G., Rantaleinen, A.L., Rogers, H.I., Sutherland, D., and van Oostdam, J. (1998) Contaminants in white sturgeon (*Acipenser transmontanus*) from the upper Fraser River, British Columbia. *Environ. Toxicol. Chem.* 19, 479.
- Martineau, D., Béland, P., Desjardins, C., and Lagacé, A. (1987) Levels of organochlorine chemicals in tissues of beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Québec, Canada. *Arch. Environ. Contam. Toxicol.* 16, 137.
- Mortensen, P., Bergman, A., Bignert, A., Hansen, H.-J., Härkönen, T., and Olsson, M. (1992) Prevalence of skull lesions in harbor seals (*Phoca vitulina*) in Swedish and Danish museum collections: 1835 - 1988. *Ambio* 21, 520.
- Muir, D.C.G., Wagemann, R., Hargrave, B.T., Thomas, D.J., Peakall, D.B., and Norstrom, R.J. (1992) Arctic marine ecosystem contamination. *Sci. Total. Envir.* 122, 75.
- Nakata, H., Tanabe, S. and Tatsukawa, R. (1995) Chlorinated hydrocarbon residues in Baikal seal (*Phoca sibirica*) from Lake Baikal: levels, patterns, and metabolism. SETAC Congress Vancouver Abstract, 240.
- Olesiuk, P.F. (1993) Annual prey consumption by harbor seals (*Phoca vitulina*) in the Strait of Georgia, British Columbia. *Fish. Bull.* 91, 491.
- Osterhaus, A.D.M.E., De Swart, R.L., Vos, H.W., Ross, P.S., Kenter, M.J.H., and Barrett, T. (1995) Morbillivirus infections of aquatic mammals: newly identified members of the genus. *Vet. Microbiol.* 44, 219.
- Rantaleinen, A.L., Ikonou, M.G., and Rogers, H.I. (1998) Lipid-filled semipermeable membrane devices (SPMDs) as traps for toxic chemicals in the lower Fraser River, Vancouver, British Columbia. *Chemosphere*
- Reijnders, P.J.H. (1986) Reproductive failure in common seals feeding on fish from polluted coastal waters. *Nature* 324, 456.
- Ross, P.S., De Swart, R.L., Addison, R.F., Van Loveren, H., Vos, J.G., and Osterhaus, A.D.M.E. (1996a) Contaminant-induced immunotoxicity in harbour seals: wildlife at risk? *Toxicology* 112, 157.
- Ross, P.S., De Swart, R.L., Van Loveren, H., Osterhaus, A.D.M.E., and Vos, J.G. (1996b) The immunotoxicity of environmental contaminants to marine wildlife: A review. *Ann. Rev. Fish Dis.* 6, 151.
- Ross, P.S., Ikonou, M.G., and Addison, R. (1997) Levels of PCBs, PCDDs and PCDFs in British Columbia harbor seals (*Phoca vitulina*) associated bioeffects. Society of Environmental Toxicology and Chemistry, San Francisco 217(Abtract).
- Skaare, J.U., Markussen, N.H., Norheim, G., Haugen, S., and Holt, G. (1990) Levels of polychlorinated biphenyls, organochlorine pesticides, mercury, cadmium, copper, selenium, arsenic, and zinc in the harbour seal, *Phoca vitulina*, in Norwegian waters. *Environ. Pollut.* 66, 309.
- Subramanian, A., Tanabe, S., Tatsukawa, R., Saito, S., and Miyazaki, N. (1987) Reduction in the testosterone levels by PCBs and DDE in Dall's porpoises of northwestern North Pacific. *Mar. Pollut. Bull.* 18, 643.
- Tillitt, D.E., Ankley, G.T., Giesy, J.P., Ludwig, J.P., Kuritamatsuba, H., Weseloh, D.V., Ross, P.S., Bishop, C.A., Sileo, L., Stromborg, K.L., Larson, J., and Kubiak, T.J. (1992) Polychlorinated biphenyl residues and egg mortality in double-crested cormorants from the Great Lakes. *Environ. Toxicol. Chem.* 11, 1281.
- Van Zorge, J.A., Van Wijnen, J.H., Theelen, R.M.C., Olie, K., and van den Berg, M. (1989) Assessment of the toxicity of mixtures of halogenated dibenzo-p-dioxins and dibenzofurans by use of toxicity equivalency factors (TEF). *Chemosphere* 19, 1881.
- West, J.E. (1997) Protection and restoration of marine life in the inland waters of Washington State. Puget Sound/Georgia Basin Environmental Report Series: Number 6, Olympia, WA, USA.